Research Article

Biosensor Design for Detection of Mercury in Contaminated Soil Using Rhamnolipid Biosurfactant and Luminescent Bacteria

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In this study, a biosensor is designed to remove mercury as a toxic metal contaminant from the soil. The rhamnolipid biosurfactant was used to extract the mercury sorbed to soil to the aqueous phase. An immobilized bioluminescent bacterium (*Escherichia coli* MC106) with pmerRBPmerlux plasmid is assisted for mercury detection. A significant decrease in luminescence level was observed in a biosensor system containing contaminated soil sample extract. The concentrations of extracting mercury are well correlated with the mercury toxicity data obtained from experimental biosensor systems according to the RBL value. The optimum aeration rate of 20 ml/min was obtained for the biosensor systems. The advantage of such a biosensor is the in situ quantification of mercury as a heavy metal contaminant in soils. Therefore, this system could be proposed as a good biosensor-based alternative for future detection of heavy toxic metals in soils.

1. Introduction

Soil pollution is defined as the existence of toxic substances at high concentrations. All soils contain various contaminants such as metals, inorganic ions, salts, PAHs, etc. These compounds are mainly formed through soil microbial activity [1]. Furthermore, various compounds discharge into the soil from the atmosphere. These contaminants are stored in plants by growing on contaminated soils [2, 3]. Metals, especially mercury, are the most essential materials that contaminate the soils. The remediation of mercury-contaminated soils is obligatory due to its high toxicity [3, 4]. Mercury poisoning is very harmful, and the impacts of mercury contact can be severe [4, 5]. Consuming the fish that is high in mercury is related to an increased danger of cancer [5, 6]. The primary sources of mercury are metal processing, mining activities, fossil fuel burning, chloride derivatives, and fertilizers [2, 3]. However, it seems to be the atmospheric precipitation is the main source of metallic pollution [7].

There are many methods for eliminating metallic contaminants, especially mercury, from soils. These methods are including the coagulation of soluble substances and adsorption on active carbon [8, 9] and the emulsification process by surfactants [9]. Physical, chemical analysis, and atomic absorption spectroscopy are commonly used to detect heavy metals in the soil [10]. Unfortunately, the analysis of toxic materials by these methods is difficult and expensive [11]. Also, the physicochemical analysis of the toxicity of the materials could not provide the information to evaluate the toxicity of the material [10, 12]. Although many methods are used for the detection of heavy metals in soil, there has been little study about microbial and biological methods. Therefore, alternative methods should be investigated. Biosurfactants are considered a possible green compound that increases the rates of heavy metals extraction from the soil to the aqueous phase [13]. Luminous bacteria are mainly defined as light-emitting microorganisms. Due to the presence of a luxCDABE gene, this group of bacteria generates luciferase enzyme and consequently produce light

in the visible spectrum. This ability has led to the use of these bacteria as whole-cell sensors for the detection of various types of environmental pollutants [14, 15]. The principle of luminescence bacteria is based on changes in the severity of light production under the influence of toxic substances [16]. Since the luminescence bacterial is directly related to cellular respiration, any inhibition of cellular metabolism due to toxicity resulted in a decrease in the emission of light in the affected cells, and it is an indication of unusual conditions. It may be the toxicity of the ecosystem [8, 14]. Recently, recombinant luminescent strains (Lux CDABE genes) were used as a detection system for environmental pollutants, especially in water and soils [17]. This detection system's advantages are comfortable performance, rapid responses, and low costs [18].

Zou et al. [19] proposed a method involving the use of pH control and rhamnolipid to enhance the efficacy of removing aging from EK correction. By cycling electrolytes, exchanging electrodes, and implementing pH electrolyte 7 of pH, with high values of 12.12%, 10.48, and 14.00, respectively, with increasing electrolyte cycling, electrode exchange was successfully improved and compared with 0.35 achieved with traditional EK treatment. The effect of adding rhamnolipid on the removal of PK EK was also investigated. In another study, Sun et al. [20] showed that a combination of EK and Fe/C-PRB is effective and promising in eliminating persistent organic pollutants (POPs) from contaminated soil by reinforcing rhamnolipid. Hansen et al. [21] produced three significant biosensor vectors to detect low mercury concentrations. These biosensors offer three different reporting gene systems, LuxCDABE, lacZYA, and GFP, with a combination of Pmer. Furthermore, the structures were placed in mini-Tn5 delivery carriers, so there is an option for Gram-negative bacteria to be used as biosensors host cells. Rasmussen et al. [22] examined two different types of soil (forest soil and beech soil) with 2.5 mg of mercury (II) in microscopes and the frequency of mercury-resistant heterogeneities. In agricultural soils, the initial concentration of available mercury was estimated at 40 mg g^{-1} . This concentration did not change during the first three days and, at the same time, increased resistance levels and decreased diversity. The available mercury concentration subsequently decreased rapidly and remained exactly above the detection level (0.2 g/g) for the remaining experiments. As a possible result of reduced mercury selection pressure, resistance and variety gradually return to preexposure values. In the soil of the beech forest, the concentration of available mercury was observed to be about 20 mg/g G1 during the experiment. This concentration does not, in any way, change the strength or variety. This study showed that measuring using a mer-Lux biosensor is a useful and sensitive tool for estimating the bioavailability available in the soil [22].

Corbisier et al. [23, 24] designed biosensors that emit light in the presence of specific metals. They used a copper biosensor (AE1239) and zinc, cadmium, and lead biosensor (AE1433) to detect the presence of available metals in fly-ashes (IFA) and soils. The produced biosensors could successfully evaluate the bioavailability of heavy metals. These biosensors were very relevant since no pretreatment of the

soils and IFA was needed. Leth et al. designed a biosensor for the detection of Cu, using luxCDABE strain from *Vibrio Fischeri* [25, 26]. Enzyme-, DNA-, immune-, whole-cell-based biosensors were designed by Turdean et al. for the detection of heavy metals such as Pb, Cd, and Ni [27, 28]. Jouanneau et al. improved heavy metal detection using different strains of *E. coli* as bioluminescence bacteria [29, 30].

Kursunlu et al. and Sulak et al. designed and prepared fluorescent alkyl-chloride boradiazaindacene (Bodipy) based sensors [31–33]. They showed that Calix [4]-B (obtained by click reaction [31] and a fluorescent Bodipy with alkyl-chloride terminal) [32], aryl-amine (containing azacrown ether ring and Bodipy) and on/off rhodamine-Bodipy (RB) [33] are appropriate sensors which could be used for high sensitivity detection of mercury ion.

Despite various studies on the extraction of mercury from contaminated soil, much of the focus has been on the use of chemical surfactants. However, the fundamental gap in the use of various methods has not yet been addressed. Thus, biosurfactant rhamnolipid, which has not been evaluated so far, was used in this study. Despite all this research, the mescore genome from *E. coli* bacteria has not been used for mercury so far, except in very few cases where soil extraction has not been fully and accurately studied. The extraction of rhamnolipid can be used to increase the extraction rate. Therefore, the current study focuses on the biosensor design for the detection of mercury toxicity using rhamnolipid biosurfactants and recombinant bioluminescent bacteria as biosensing systems.

2. Materials and Methods

2.1. Materials

2.1.1. Soil Preparation. Figure 1 shows the image of the light production of bioluminescence bacteria during the bacteria growth. The soil sample was collected from a garden located in the Ahar industrial city, East Azerbaijan, Iran. The selected soil sample was sieved at 1 mm to remove the coarse elements before the tests. The soil heavy metal contaminants are measured using atomic absorption spectroscopy. For evaluating the mercury toxicity, 100 ppm of mercuric chloride is added to 50 g of the soil sample at constant pH (7.2). The analyzed data are presented in Table 1.

2.1.2. Production of Biosurfactants. The rhamnolipid biosurfactant is applied as an emulsifier for improving the efficiency of mercury extraction from the soil into the aqueous phase. Rhamnolipid was a mixture of mono- and dirhamnolipid at an approximate ratio of 1:1. The CMC (Critical Micelle Concentration) and HLB (Hydrophilic Lipophilic Balance) value of produced rhamnolipid were 180 mg/l and 9.5, respectively. The producing procedure for rhamnolipid biosurfactant is mentioned in our previous work [34]. The biosurfactant was characterized using TLC spectroscopy. Figure 2 shows the results of TLC spectroscopy for mono- and dirhamnolipid and then compared with the standard data [35]. Also, Table 2 shows the retention

FIGURE 1: Luminescent bacteria growing on a nutrient agar plate in the dark room.

TABLE 1: Soil characteristics and analysis.

Parameter	Concentration (%)
Fine sand	23
Large sand	72
Silt	2.5
Clay	2.5
Organic compounds	3
Cd	0.23
Ni	1.2
Zn	0.7
Cr	0.8

FIGURE 2: TLC of the mono- and dirhamnolipid: (a) produced; (b) reported in research studies.

TABLE 2: Retention factor of produced rhamnolipid.

R_f values	R_{f2} (c/a)	R_{f1} (b/a)
Produced	0.9	0.45
Reported	0.68	0.35

factor (R_f) of produced rhamnolipid. The dried transparent glass beads with the diameter range of 300–500 μm were purchased from Zhejiang Pujiang Jingsheng Crystal Co., Ltd, China. Polypropylene tubes were purchased from India MART Co., Noida, India. All materials for the

immobilization process of bacteria were purchased from Sigma Aldrich Co., Germany.

2.2. The Immobilization of Luminescence Bacteria.

Polypropylene tubes' outer diameter and length are 8.5 and 10 mm, respectively. The polypropylene tubes and glass beads were washed with water, autoclaved, and completely dried in a drying oven before use after autoclaving was used for immobilization of bioluminescence bacteria. Furthermore, the dried transparent glass beads with a diameter range of 300–500 μm were assisted in increasing the sensitivity of the biosensor. Each polypropylene tube contains 0.1 g of glass beads. The bioluminescent bacterium is a recombinant pmerRBPmerlux plasmid was transformed into the *Escherichia coli* strain MC 106 1 introduced by Rantala et al. At the concentration of the cells was between 1×10^9 and 2×10^9 cells/tube, the high response of the *E. coli* cells was obtained [36]. *Escherichia coli* MC106 cells were grown at 37°C in a shaking incubator (Model: GFL 3032, Germany) at 200 rpm. The incubator contains Luria Bertani (LB) (Sigma Aldrich Co. Germany) medium supplemented with 50 mg/l streptomycin. The optical density should reach 4 during the incubation process. After incubation, 100 ml of culture medium was centrifuged at 350 rpm at room temperature for 20 min. The collected cells were suspended in 1 ml of fresh LB medium. After autoclaving, the mixture of 15 g/l micro agar (Sigma Aldrich Co. Germany) and 25 g/l LB medium should be maintained at 40°C in the water bath during the immobilization procedure. Pipes containing 0.1 g of glass beads were filled with 200 μl of cell mixture. The cellular mixture in the tubes was stabilized at 25°C.

2.3. Bioreactor Design.

The bioreactor setup is shown in Figure 3. As shown in this figure, a stainless-steel bioreactor ($V=100$ ml) equipped with a water jacket was used to maintain the inside temperature at a constant value. The bioreactor was filled with 25 ml LB medium. The sparge tube is connected to the inlet of the reactor vessel for preparing fresh air. Furthermore, two connections were available for gas venting and injection of the toxic samples (mercury). A fiber-optic probe was attached to the biosensor kit. A sensitive luminometer (Model: Smart Line TL Tube Luminometer, UK) was joined to the end of a fiber-optic probe to measure the bioluminescence (BL) signal from the immobilized cells. This system was connected to a PC in order to collect real-time data (Figure 3). The schematic of the biosensor kit is shown in Figure 4.

2.4. Extraction of Mercury Using Rhamnolipid.

The soil samples were washed with fresh water, sterilized, and dried in an oven. 5 g of the soil samples (contain 200 ppm of mercury chloride) was mixed with 50 ml of rhamnolipid with different concentrations (0.5, 1, 1.5, 2, 2.5, and 3 g/l). To increase the efficiency of mercury extraction, soil slurry samples were mixed at 200 rpm and room temperature (25°C). After the extraction process, samples were centrifuged at 345 rpm to remove particles. The supernatants were

FIGURE 3: Biosensor system used in this study.

FIGURE 4: Schematic of the biosensor kit.

analyzed by AAS (Atomic Absorption Spectroscopy) to determine the mercury concentration.

2.5. Biosensor Operation. Different concentrations of the extracted mercury were prepared and stored in glass vials. After the bioluminescent (BL) test of the prepared biosensor, 5 ml of the mercury was injected into the biosensor system. Rhamnolipid biosurfactant is a nonvolatile and nontoxic emulsifier. This will decrease the chance of mercury evaporation during the extraction process. Therefore, the developed biosensor system has good sensitivity.

3. Results and Discussion

3.1. Effect of Rhamnolipid Concentration on Mercury Extraction. Rhamnolipid biosurfactant as an emulsifier was used to increase the solubility of mercury in the aqueous phase. Because of the nontoxicity of rhamnolipid, the sensitivity of the biosensor was not affected during increasing the rhamnolipid concentration. Figure 5 shows the effect of rhamnolipid concentration on mercury extraction. The concentration of mercury in the aqueous phase has increased significantly with the increase in rhamnolipid concentration. This is due to the anionic biosurfactant and

FIGURE 5: Effect of rhamnolipid concentration on mercury extraction.

FIGURE 6: Extracted and solid phase during the metal removing process (rpm = 200, $T = 25^{\circ}\text{C}$).

cationic metal interaction. The biosurfactant CMC (Critical Micelle Concentration) value of the rhamnolipid is about 100 mg/l [37]. The CMC of the biosurfactant can be raised by increasing the rhamnolipid concentration. Therefore, the mercury concentration is elevated in the aqueous phase. After 30 min of the extraction process of the rhamnolipid concentration of 3 g/l, the mercury concentration in the aqueous phase reached 150 ppm (Figure 5). Figure 6 shows the extracted and solid phase during the metal removal process.

Figure 7 shows the concentration of extracted mercury via extraction time at the concentration of 3 g/l of rhamnolipid. According to Figure 7, about 75% of mercury was extracted from the contaminated soils at the highest concentration (3 g/l) of rhamnolipid. The yield of mercury extraction increases with increasing the extraction time. This happened due to an increase in the rate of micelle formation, which will enhance the mass transfer rate between the mercury ions and rhamnolipid biosurfactant. Figure 8 shows the microscopic image of the extraction process and the micelle formation of rhamnolipid. The aqueous phase penetrated the contaminated soil by rhamnolipid micelle formation, and the extraction process goes well.

3.2. Determination of the Optimum Aeration Rate. Immobilized *E. coli* cells in the biosensor kits floated in the LB. Due to lack of oxygen, bioluminescence bacteria activity is affected and light production is affected. The lack of a

FIGURE 7: Concentration of extracted mercury via time of extraction at the concentration of 3 g/l (rpm = 200, $T = 25^{\circ}\text{C}$).

sufficient oxygen supply would affect the BL of the immobilized cells since it is needed for the production of light. The designed biosensor was tested for three different aeration values (no aeration, 20-, and 40-ml air/min). Figure 9 shows how the aeration rate in BL affects the immobile cells. As shown in the fig, the BL values of the samples with no aeration and 40 ml air/min both decreased close to zero after 50 min. However, the other sample with an airflow rate of 20 ml/min reached the constant BL value of 200000 after 30 min, and this value was constant for more than 50 min. The results showed that *Escherichia coli* needs enough oxygen concentration for growing. The lack of adequate oxygen slows down the growth of immobilized bacteria. On the

FIGURE 8: Microscopic image of the extraction process and micelle formation of rhamnolipid.

FIGURE 9: Effect of the aeration rate on the BL of the immobilized cells ($T = 37^{\circ}\text{C}$).

other hand, the excess oxygen causes a decrease in the BL of the immobilized cells and inhibit bacterium growth. The optimum airflow rate of 20 ml/min was used for the experiments.

3.3. Effect of Mercury Concentration on the Bioluminescence Level of a Biosensor System. In this step, the extracted mercury in the micelle of the rhamnolipids was injected into the test reactor and biosensor kit. The effect of mercury concentration on the bioluminescence level of the biosensor system is shown in Figure 10. As shown in this figure, The Bioluminescence Level decreased quickly for the first time according to the mercury concentration, and then, after 30 min from injection decreased *E. coli* cells continued to slowly decrease after the initial drop in the BL value. The effect of mercury concentration on the bioluminescence level of the biosensor system is shown in Figure 10. As the concentration of the injected mercury is increased, the rate of BL value of luminescence bacteria decreased. The mercury toxicity causes inhibition in bacterial growth that resulted in decreasing the bioluminescence level of the bacteria [38]. As is shown in Figure 10, the concentrations of extracted mercury are well correlated with the mercury toxicity data

FIGURE 10: Effect of mercury concentration on bioluminescence level of biosensor system for 30 in after injection (aeration rate: 20 ml/min, $T = 37^{\circ}\text{C}$).

FIGURE 11: The variation of RBL with time for the biosensor system for different mercury concentrations (Aeration rate: 20 ml/min, $T = 37^{\circ}\text{C}$).

achieved by a biosensor system, according to Figures 5 and 10.

3.4. Bioluminescence Bacteria Performance. Inhibition of cellular metabolism due to the toxicity of mercury was observed in *Escherichia coli* MC106 cells. Similar concentrations of rhamnolipid without mercury were injected into the control biosensor system. The toxicity is determined using the equation below as Relative Bioluminescence ($\text{RBL} = \text{BL in the test system} / \text{BL in the control system}$). Figure 11 shows the RBL variation with time in the biosensor system for different concentrations of the extracted mercury. The RBL value of the different concentration of mercury was measured as 1, 0.9, 0.6, 0.35, and 0.1 ± 0.05 for 0, 5, 10, 15, and 20 ppm of mercury in the biosensor system at 30 min after injection. Using this value and Figure 11, the mercury concentrations could be calculated easily. A reduction in RBL of the system is seen due to the inhibition of the bacterial metabolism caused by toxic ions of extracted mercury [39].

4. Conclusions

In this work, a setup is designed to investigate the performance of *Escherichia coli* MC106 bacteria as a biosensor to detect mercury in contaminated soil. The rhamnolipid biosurfactant assisted in increasing the rate of mercury release from the soil to the aqueous phase. of the nontoxicity of rhamnolipid, it does not affect the accuracy of mercury concentration measurements. Results showed that the strong anion-cation interaction between rhamnolipid and mercury would cause an increase in mercury concentration in the aqueous phase by increasing in rhamnolipid concentration. The optimum airflow rate of 20 ml/min was estimated and used for the experiments. *Escherichia coli* MC106 cells show a reduction in its bioluminescent level with inhibition of the bacterial metabolism caused by toxic chemicals. The results that approved the designed biosensor are also able to detect the toxicity of mercury. Therefore, this proposed system has high potential as an in situ inspection technique because of its cheapness, simplicity, and rapid responses.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest reported in this paper.

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